

STUDIES ON THE ROLE OF THE CORPUS ALLATUM IN THE ERI-SILKWORM, *PHILOSAMIA CYNTHIA RICINI*¹

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The corpus allatum of insects has two known functions. In the developing insect, it furnishes a hormone which, in collaboration with the growth and differentiation hormone of the prothoracic glands (or their homologues), brings about larval molts. In the adult female, presumably the same corpus allatum hormone stimulates gonadal development, especially yolk deposition in the eggs. The latter effect has been demonstrated in a variety of species representing, among others, Orthoptera (Pfeiffer, 1939; Scharrer, 1946), Hemiptera (Wigglesworth, 1936), and Diptera (Thomsen, 1940, 1942; Vogt, 1941, 1943; Day, 1943). On the other hand, the adult ovaries of several representatives of Lepidoptera tested proved independent of the corpus allatum hormone (Bounhiol, 1942; Fukuda, 1944; Williams, 1946).

In another lepidopteran, the Eri-silkworm, *Philosamia cynthia ricini*, the corpus allatum of the newly emerged moth is 20 times larger than that of the last instar larva, an observation which suggests that this gland is functionally active in the adult of this species. In the course of experiments designed to demonstrate this physiological activity in adult *Philosamia*, a new role of the corpus allatum was discovered.

MATERIAL AND METHODS

Larvae of *Philosamia* were reared at around 25° C. Pupae from which the brain had been removed not later than 22 hours after pupation (artificially induced diapause) were used as test animals. Four to 6 corpora allata from donors of different stages were implanted into these diapausing pupae through a small hole in the dorsal integument of the second or third abdominal segment. The hole was then covered with a piece of integument and the wound was coated with melted paraffin. In some additional experiments, brains were implanted together with corpora allata; in others, corpora cardiaca were added, since they are known to store neurosecretory material originating in the brain. Following the implantation, the specimens were kept again at about 25° C. and were examined at appropriate intervals.

RESULTS

1. *Implantation of corpora allata from adult donors*

Implants of corpora allata from male or female donors whose adult age was 1-2 days, into diapausing pupae that had been deprived of their brains for two months,

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were effective in 9 out of 10 cases (Table I). Within 22–32 days after implantation the hosts underwent an additional pupal molt. These animals were unable to shed the old pupal cuticle by themselves, but molting fluid was present so abundantly that the old cuticle could be easily removed by forceps. The new pupal skin thus exposed was of normal color in the posterior half of the animal, but it appeared yellowish white in the anterior part. The imaginal discs of wings, antennae, and legs showed a very slight development toward the adult form while other organs displayed no sign of adult differentiation.

This result reveals two important effects of the corpus allatum of *Philosamia*: (1) the implants must have furnished juvenile hormone since the molt following their implantation was pupal rather than adult. This effect is in keeping with the known role of the corpora allata in a variety of insect species. (2) The implants, in addition to the juvenile hormone, must have furnished a principle which initiated molting in a diapausing host deprived of its brain. It was concluded that this molt-inducing hormone originated in the neurosecretory cells of the brain of the donor and was stored in its corpus allatum. An axonal transport of neurosecretory ma-

TABLE I
Implantation of endocrine organs isolated from adults

Endocrine organ	Number of implanted organs	Number of experimental specimens	Number of deaths or undeveloped cases	Number of adults	Number of second pupal instars
Corpus allatum	4–6	10	1	0	9 (90%)
Corpus cardiacum	4–6	10	0	1* (10%)	0
Brain-cardiaca-allata complex	3	15	3	0	12 (80%)

* The interval needed for its development was abnormally long.

terial produced in the insect protocerebrum has already been demonstrated in earlier investigations (Scharrer and Scharrer, 1944; Scharrer, 1952; M. Thomsen, 1954, and others). In many species, the neurosecretory material can be traced only as far as the corpora cardiaca which in these forms are considered as the main storage and release center of neurosecretory hormones. Therefore, corpus cardiacum implants and brain implants, either alone or in combination with corpus allatum implants, also were tested.

When four to six corpora cardiaca were implanted into each of ten diapausing pupae, only one of the recipients emerged 47 days later, an interval much longer than that normally required for adult development. The other nine hosts remained unchanged. This result demonstrates at best only a minor role of the corpus cardiacum of *Philosamia* as a storage center for neurosecretory material.

Each of 15 diapausing pupae (417 days after their brain was extirpated) received three complexes of brain-corpora cardiaca-allata plus subesophageal ganglion. Three animals died. Twelve of the hosts pupated again within three weeks after implantation; none proceeded to become an adult moth. These results do not differ from those after the implantation of corpora allata alone.

2. *Implantation of corpora allata from pupal donors*

The pupae which furnished the corpora allata in this series had passed from 11 to 13 days in the pupal state. Again each of the diapausing hosts received six corpora allata. Seventeen out of 22 pupae thus operated upon differentiated quite normally into moths within 25 days after the implantation (Table II). The remaining five hosts remained pupae or died before showing any positive result. It is of interest that none in this group underwent a second pupal molt. Thus the result differs from that of the previous experiment in which adult corpus allatum implants had been used. One must conclude that pupal corpora allata contain only the hormone which stimulates the prothoracic glands, but are devoid of appreciable amounts of juvenile hormone.

The addition of pupal brains and corpora cardiaca to corpus allatum implants did not alter the outcome of the results. Twenty-one out of 22 diapausing animals

TABLE II
Implantation of corpora allata isolated from pupae and larvae

Endocrine organ	Number of implanted organs	Number of experimental specimens	Number of deaths or undeveloped cases	Number of adults	Number of second pupal instars
Pupal donors:					
Corpus allatum	6	22	5	17 (77.3%)	0
Brain-cardiaca-allata complex	3	22	1	21 (95.4%)	0
Brain	3	23	4	19 (82.6%)	0
Larval donors:					
Corpus allatum (5th instar)	6	24	0	5* (20.8%)	19 (79.2%)
Corpus allatum (4th instar)	6	27	6	0	21 (77.8%)

* One specimen required an abnormally long interval.

receiving these grafts emerged after about 25 days; the remaining one died. Thus, none of these animals underwent an additional pupal molt.

In another group of test animals each of which received three pupal brains, emergence occurred after the same period of time in 19 out of 23 specimens. These results show that (a) implants of either pupal brains or pupal corpora allata furnish the hormone necessary for the initiation of adult differentiation, and (b) pupal corpora allata do not contain appreciable amounts of juvenile hormone.

3. *Implantation of corpora allata from larval donors*

Among 27 test animals which received corpora allata removed from fourth instar caterpillars two days before the next molt, 21 underwent a second pupal molt within 11 to 14 days. None showed adult differentiation. The result was somewhat different when the donors were fifth instars which had just entered the spin-

ning stage. In this group 19 out of 24 test animals had another pupal molt while four became adult moths after a normal, and one after a prolonged, interval of time. It seems that in the last mentioned five cases the corpora allata had already ceased to secrete juvenile hormone.

4. Extirpation of corpora allata from pupae

Since the preceding experiments had demonstrated the presence of juvenile hormone in the corpora allata not only of larval but also of adult *Philosamia*, the question arose which role is played by these glands in the imago. A possible control over gonadal activity was tested by removing the corpora allata from pupae not older than 40 hours which were then allowed to complete their adult development. Twelve allatectomized specimens did not differ essentially from 20 sham operated controls. In each group about the same number of eggs became mature (Table III). In other

TABLE III
Comparison of egg development in allatectomized and control females

	Number of specimens examined	Average number of eggs		
		Mature	Immature	Total
Allatectomized	12	128	150	278
Control	20	154	131	285

words, in *Philosamia* ovarian function seems to be independent of the corpora allata. Future tests with biochemical methods will be needed to show whether or not the corpora allata in this species have a metabolic function.

DISCUSSION

The present experiments have revealed that in *Philosamia* brainless pupae can be induced to molt by the implantation of corpora allata. Depending on the stage of the donor, the molt caused may or may not be coupled with adult differentiation. Larval and adult corpora allata furnish enough juvenile hormone to render the ensuing molt of the test animal a second pupal molt. By contrast, pupal corpora allata lack effective doses of juvenile hormone. The type of molt occurring is, however, of less interest than the fact that molts can be induced at all by corpus allatum implants in cases where they would otherwise not occur. While it has been known for some time that corpora allata from larval and adult donors can furnish juvenile hormone, the present study offers the first evidence that corpus allatum implants can induce molting. Theoretically, the molt-inducing hormone present in the corpus allatum implants used in our experiments either could have originated in the corpora allata themselves, or it could merely have been stored there. The first possibility seems less likely. The reasons for assuming the second mode of action are as follows. In *Philosamia* as well as other forms of insects, neurosecretory cells of the brain are known to furnish a hormone which stimulates the prothoracic glands into releasing a molt-promoting hormone. It is also known that this neurosecretory

material is transported along axons and stored at some distance from the site of origin. In a variety of species the storage and release center is the corpus cardiacum. In some species, including *Philosamia cynthia*, neurosecretory material has been observed to enter also the corpus allatum. However, the possibility that this gland stores neurosecretory material in appreciable amounts has never been tested experimentally with positive results. So far, the presence of neurosecretory material within the corpus allatum tissue has been interpreted as a possible morphological indication for the existence of an allatotropic action on the part of neurosecretory cells (E. Thomsen, 1954). The present study neither contradicts nor supports this view. However, judging from the result with pupal donors of *Philosamia*, juvenile hormone can be absent in corpora allata in which brain hormone is known to be stored. Therefore, one would have to assume that corpus allatum cells do not necessarily respond under all circumstances to stimulation by an "allatotropic hormone." Furthermore this factor may or may not be identical with the molt-inducing hormone.

The present study offers evidence that implants of corpora allata in *Philosamia* furnish brainless pupae with a sufficient amount of neurosecretory material to induce them to molt. It does not prove that in the intact animal the corpus allatum tissue serves as the main storage and release center of a hormone produced by the brain. The possibility exists that neurosecretory material which reaches the organ via the nervi corporis allati accumulates within the corpus allatum in gradually increasing amounts without being given off into the circulation. This situation would perhaps be comparable to the accumulation of juvenile hormone in the abdomen of adult males of *Platysamia* (Williams, 1956). Further experiments will be needed to determine whether in species such as *Philosamia* with inconspicuous corpora cardiaca the corpora allata indeed take over the main storage and release function.

The experimental demonstration of the presence of molt-promoting hormone in the corpora allata of *Philosamia* is paralleled by morphological data showing the existence of a corresponding neurosecretory pathway. The presence of neurosecretory material in the nervi corporis allati has been observed in *Bombyx* (Bounhiol, Gabe and Arvy, 1953, 1954; Kobayashi, 1957) as well as *Philosamia* (unpublished observations of the authors).

Whatever the mechanism of release of neurosecretory hormones under normal physiological conditions, the fact remains that, with the exception of the pupal stage, the corpora allata of *Philosamia* contain two hormones controlling post-embryonic development, the "prothoracotropic hormone" of neurosecretory origin and the "juvenile hormone" produced by the corpus allatum cells themselves.

SUMMARY

1. Pupae of *Philosamia cynthia ricini* in which diapause had been artificially induced by the removal of the brain, served as test animals for the effects of corpus allatum implants. Four to six corpora allata from donors in different stages induced molting in hosts which otherwise would have remained pupae. It was concluded that in *Philosamia* the corpus allatum, in addition to producing juvenile hormone, contains an appreciable amount of molt-inducing hormone furnished by neurosecretory cells of the brain. The interpretation is supported by the existence, in *Philosamia* as well as other insect species, of a neurosecretory pathway which links

the secretory part of the brain with the corpora cardiaca-allata and which permits the storage of hormones produced in the brain at some distance from the cells of origin. While in most species studied so far the main storage center is the corpus cardiacum, this role may have been taken over by the corpus allatum in *Philosamia*.

2. As might be expected, the molt induced may or may not be coupled with adult differentiation depending on the stage of the donor. Implants of corpora allata from adult or fourth instar larval donors caused an additional pupal molt because, in addition to molt-inducing hormone, they also supplied juvenile hormone to the host. By contrast, implants from pupal donors contained no appreciable amount of juvenile hormone with the result that they brought about an imaginal molt. Some of the fifth instar implants had the same effect as those from pupae, while others acted like tissues from fourth instars. It seems that during the fifth larval stage the change from activity to temporary inactivity of the corpus allatum cells occurs gradually. Thus implants of larval and adult corpora allata furnish two hormones controlling post-embryonic development, while pupal corpora allata contain only one, namely, the neurosecretory material derived from the protocerebrum.

3. Even though the presence of corpus allatum hormone has been demonstrated in glands from adult donors in the present experiments, the role normally played by this hormone in the adult moth is still unknown. Extirpation of corpora allata from female pupae of *Philosamia* did not prevent egg maturation in the resulting moths.

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